DATA EVALUATION RECORD

TRIFLUMEZOPYRIM

STUDY TYPE: COMBINDED CHRONIC TOXICIY/CARCINOGENICITY -RAT (OCSPP 870.4300)

MRID 49382173

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 S. Crystal Drive
Arlington, VA 22202

Prepared by
Summitec Corporation
9724 Kingston Pike, Suite 602
Knoxville, Tennessee

Task 6-169

Initial Reviewer:
Hoban, D., DuPont Author

Signature: D. Hoban AE
Date: 07/22/2016

Secondary Reviewers:
Jess Rowland, M.S.

Signature: Jess Rowland AE
Date: 07/22/2016

Robert H. Ross, M.S., Program Manager

Signature: Robert H Ross AE
Date: 07/22/2016

Quality Assurance:
Angela M. Edmonds, B.S.

Signature: Angela M. Edmonds
Date: 07/22/2016

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EPA Reviewer: Monique M. Perron, Sc.D. **Signature**: _____
Risk Assessment Branch I, Health Effects Division (7509P) **Date:** _____
EPA Secondary Reviewer: Anwar Y. Dunbar, Ph.D. **Signature**: _____
Risk Assessment Branch I, Health Effects Division (7509P) **Date:** _____

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DATA EVALUATION RECORD[1]

STUDY TYPE: Combined dietary chronic toxicity/carcinogenicity - Rat; OCSPP 870.4300 [§83-5]; OECD 453.

PC CODE: 129210 **DP BARCODE:** D432127

TEST MATERIAL (PURITY): Triflumezopyrim technical (Purity 99.4% a.i.)

SYNONYMS: DPX-RAB55; 2,4-Dioxo-1-(5-pyrimidinylmethyl)-3-(3-(trifluoromethyl)-phenyl)-2H-pyrido(1,2-a)pyrimidinium inner salt

CITATION: Papagiannis, C. (2015); Triflumezopyrim (DPX-RAB55) technical: Combined chronic toxicity/oncogenicity study 2-year feeding study in rats. MPI Research, Mattawan, Michigan, USA. DuPont Report No. 34939. Study date November 18, 2015, MRID 49382173.

SPONSOR: E. I. du Pont de Nemours and Company, Wilmington, Delaware 19898

EXECUTIVE SUMMARY:

In a 2-year chronic toxicity and carcinogenicity feeding study (MRID 49382173), triflumezopyrim was administered to male and female CD® [Crl:CD(SD)] rats (80 rats/sex/concentration) at concentrations of 0, 100, 500, 2000, and 8000 ppm. The overall (Week 1-104) mean daily intakes for male rats were 0, 3.03, 15.92, 70.55, and 283.83 mg/kg bw/day, respectively and 0, 3.23, 17.34, 73.80, and 395.88 mg/kg bw/day, respectively, for females. Ten rats per group were sacrificed after approximately one year and all surviving rats were sacrificed after approximately 2 years. Parameters evaluated included body weight, body weight gain, food consumption, food efficiency, clinical signs, clinical pathology, ophthalmology, serological health screen, organ weights, gross and microscopic pathology.

No treatment-related effects were seen on survival, clinical signs of toxicity, or clinical pathology in either sex at any dose level. Significant decreases in absolute body weights were seen at 2000 ppm for females only (3% – 23%), and 8000 ppm for both sexes (9% – 25% for males, and 12% – 45% for females respectively). Relative (not absolute) liver weights were increased in males and females at 2000 and 8000 ppm dose groups at the 1-year interim sacrifice and only in females at termination at these dose levels. At 2000 ppm, only females had an increased incidence of hepatocellular hypertrophy at terminal sacrifice (7% vs. 0% in the control). At 8000 ppm, increased incidences of hepatocellular hypertrophy were observed in both sexes (10% vs. 0% in controls in males; 67% vs. 0% in the control in females) at terminal sacrifice. An increase in bile duct hyperplasia was observed in males at

[1] This DER was generated by modifying the study summary in a Tier II document (MRID 49382105).

2000 ppm and 8000 ppm (63% and 77% vs. 47% in the control, respectively) at terminal sacrifice. Also seen in the liver at 8000 ppm were increases in focal cystic degeneration males only (41% vs. 24% in the control) and individual cell necrosis in females only (4% vs. 0% in the control).

In addition to the liver observations, non-neoplastic lesions of the testes of male rats and uterus of female rats were observed at 8000 ppm. Interstitial cell hyperplasia of the testes was seen at termination in males at 8000 ppm (23% vs. 4% in controls), but was not associated with organ weight changes or interstitial cell tumors. Uterine lesions included dilation/inflammation/hyperplasia (9% vs. 0% in controls), cystic endometrial hyperplasia (11% vs. 1% in controls), and squamous cell hyperplasia (9% vs. 3% in controls).

There were no increases observed in any tumor type among males. In females, there was an increase in squamous cell carcinomas, adenocarcinomas, and granular cell tumors in the uterus at 8000 ppm. There was also a slight increase in the incidence of hepatocellular adenomas in females at this dose.

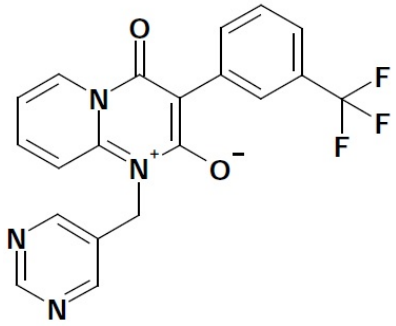
The No-Observed-Effect-Level (NOAEL) for Sprague Dawley rats was 500 ppm (15.92 mg/kg bw/day for male rats and 17.34 mg/kg bw/day for female rats). The Lowest-Observed-Effect-Level (LOAEL) was 2000 ppm (70.55 mg/kg bw/day for males and 73.80 mg/kg bw/day for females) based on the significant decreases in absolute body weight in females and increased incidence of bile duct hyperplasia in males.

At the highest dose tested (8000 ppm), decreased absolute body weight in both sexes, increased relative liver weights in females, and additional histopathological lesions in the liver (both sexes), testes, and uterus were also observed.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Triflumezopyrim technical

Lot/Batch #:	RAB55-037
Purity:	99.4%
Description:	Neutral yellow powder
CAS #:	1263133-33-0
Stability of test compound:	The 17-day room temperature stability of the formulations from 100 to 20,000 ppm in the diet had been established in a previously conducted study.
Structure:	

2. Vehicle: Untreated diet

3. Test animals:

Species:	Rat
Strain:	Crl:CD (SD)
Age at dosing:	Approximately 7 weeks old
Weight at dosing:	203–273 g for males; 127–193 g for females
Source:	Charles River Laboratories, Inc., Portage, Michigan.
Acclimation period:	14 days
Diet:	PMI® Nutrition International, LLC Certified Rodent Lab Diet® (#5002), *ad libitum*, except during designated periods. During the test period, test substance was incorporated into the feed of all animals except controls.
Water:	Tap water, *ad libitum*
Housing:	Animals were pair-housed in solid bottom cages with nonaromatic bedding and enrichment.

4. Environmental conditions:

Temperature:	20–26°C
Humidity:	30–70%
Air changes:	Not reported
Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN

1. In-life initiated/completed: 05/06/2012 to 04/06/2014

2. Animal assignment and treatment: Groups of male and female 70 CD-1 rats (80/sex/concentration) received triflumezopyrim in feed at 0, 100, 500, 2000, and 8000 ppm for two years. Animals were assigned to dose groups by computerized, stratified randomization so that there were no statistically significant differences among group body weight means within a sex. A control group received untreated diet.

Table 1. Study design: Combined Chronic Toxicity/Carcinogenicity Study in Rats Fed Triflumezopyrim.

Test Group	Concentration in diet (ppm)[a]	Dose to animal (mg/kg bw/day) [b] (Males / Females)	No. of Animals (Males)	No. of Animals (Females)
1	0 (control)	0 / 0	80	80
2	100	3.03 / 3.23	80	80
3	500	15.92 / 17.34	80	80
4	2000	70.55 / 73.80	80	80
5	8000	283.83 / 395.88	80	80

Table taken from pages 25 and animal doses were taken from section 4.2.5 of the study report (MRID 49382173).
[a] An interim necropsy was conducted on 10 animals/sex/concentration at 1 year.

3. **Dose selection:** The doses for this were selected based on the results of the 13-week study in rats in which male and female CD-1 rats were fed triflumezopyrim at concentrations of 0, 100, 400, 1500, and 6000 ppm for 13 weeks. No treatment-related effects were seen on the following parameters: survival, clinical signs, ophthalmoscopic evaluations, coagulation, clinical chemistry, urinalysis, or macroscopic evaluations. The NOAEL was 1500 ppm (63.86 mg/kg bw/day in males and 74.26 mg/kg bw/day in females), and the LOAEL was 6000 ppm (257.09 mg/kg bw/day in males and 278.14 mg/kg bw/day in females) based on adverse effects on mean body weight and food consumption in both sexes (MRID No.49382162).

4. **Diet preparation and analysis:** The test substance was added to the rodent diet and thoroughly mixed for 30 minutes. Control diets were mixed for the same period of time. All diets were prepared weekly and stored at room temperature until used. The homogeneity and concentration of triflumezopyrim in the dietary mixtures were checked by analysis using HPLC. Homogeneity analyses samples were collected before dosing (Week -1) and concentration analyses samples were collected during Weeks 1, 13, 26, 39, 52, 65, 78, 91, 92, and 104.

Results:

Homogeneity analysis: The test substance was homogeneously mixed at target concentrations in the diet. Pretest homogeneity analyses were within the expected range of <15% relative standard deviation (%RSD) when blended for 10 minutes at 100, 500, 2000, and 8000 ppm. In addition, the average percent nominal concentrations ranged between 97.5 to 104.2%. In these cases, the %RSD ranged from 2.860 to 11.817. No test article was found in the control diet.

Stability analysis: The concentration analyses at 100, 500, 2000 and 8000 ppm were within the expected range of ±20% of the nominal concentration at the analyzed intervals (Weeks 1, 13, 26, 39, 52, 65, 78, 91/92, and 104), with three exceptions at the lowest concentration of 100 ppm. At weeks 65, 91, and 104, the low concentration of 100 ppm did not meet the acceptance criterion for % RSD (≤ 15%) based on respective of %RSDs of 26.892, 22.644, and 20.781%. In all cases, the backup samples were analyzed, resulting in respective in respective %RSDs of 18.312, 17.989, and 13.192 (within criterion for the 13.192 value). An assignable cause for the high %RSD values was not determined for Weeks 65 and 104.

Concentration analysis: Overall, the average percent of the nominal concentrations ranged between 86.2 and 199.6%.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

5. Statistics:

Table 2. Statistics: Combined Chronic Toxicity/Carcinogenicity Study in Rats Fed Triflumezopyrim.

Parameter	Preliminary test	Method of statistical analysis	
		If preliminary test not significant	If preliminary test was significant
Body weight, Body weight gain, Food consumption, Hematology (except leukocyte counts), Coagulation Clinical chemistry, Organ weights (absolute and relative to body and brain weights)	Levene's test for homogeneity	One-way analysis of variance followed by Dunnett's test	Welch's t-test with a Bonferroni correction
Leukocyte counts (total leukocyte counts and differential leukocyte counts)	Log transformation followed by Levene's test for homogeneity	One-way analysis of variance followed by Dunnett's test	Welch's t-test with a Bonferroni correction
Food efficiency Urinalysis (urine volume, specific gravity and pH)	Rank transformation Levene's test for homogeneity	One-way analysis of variance followed by Dunnett's test	Welch's t-test with a Bonferroni correction
Mortality	None	Kaplan-Meier product-limit method. An overall test comparing all groups was conducted using a log-rank test	
Tumor data	None	Tumor incidence data were analyzed using both survival adjusted and unadjusted tests. The unadjusted tests were based on the incidence and number of sites examined for each tumor type. The Cochran-Armitage trend test was calculated and Fisher's exact test was used to compare each treatment group with the control group. The survival adjusted test was conducted according to the prevalence/mortality methods described by Peto *et al.*	
Non-tumor microscopic pathology data	None	Fisher's exact test and Cochran-Armitage trend test	

Significance was judged at $p < 0.05$ and $p < 0.01$. Separate analyses were performed on the data collected for each sex. The Reviewer considers the statistical analyses appropriate.

C. METHODS:

1. **Observations:** Animals were observed at least twice daily for mortality and morbidity; beginning at Week 53, a third daily cageside observation was conducted. Animals received a detailed clinical examination prior to study start, then weekly. On occasion, veterinary consultations were conducted during the course of the study. All treatments/ recommendations and observations were recorded. Animals were examined at least weekly for clinical signs of toxicity.

2. **Body weights:** All animals were weighed three days after receipt, on Day -1, on Day 1, and then once per week.

3. **Food consumption and compound intake:** Food consumption was measured and recorded for each animal weekly during the first 13 weeks and every other week (14-day intervals) thereafter. Food consumption was recorded for each cage over the weighing interval and divided by the number of animals. Food efficiency and daily intake were calculated from food consumption and body weight data.

4. **Ophthalmolscopic examinations:** Ophthalmoscopic examinations were conducted on all animals prior to dosing and on all surviving animals prior to the interim necropsy and prior to the terminal necropsy.

5. **Hematology, clinical chemistry, and urinalysis:** Blood and urine samples were collected from all animals approximately 3, 6, and 12 months after initiation of the study. Animals had access to water but were fasted overnight prior to sample collection. At the interim (12-month) sacrifice, blood and urine were collected, and bone marrow smears were prepared. Hematology, clinical chemistry, coagulation, and urinalysis were performed on the samples. Blood smears were prepared on all surviving animals at 12, 18, and 24 months and from all animals euthanized *in extremis*. Manual differential white blood cell (WBC) counts were evaluated from all animals euthanized *in extremis* and from control and high-concentration animals from the 24-month terminal necropsy. No additional manual differential WBC counts (%) were evaluated from rats at other time points or from lower dose groups, as no test substance-related effects were noted for the intervals/groups evaluated.

a. Hematology:

Hematocrit (HCT)*	Leukocyte differential count*
Hemoglobin (HGB)*	Mean corpuscular HGB (MCH)*
Leukocyte count (WBC)*	Mean corpuscular. HGB conc.(MCHC)*
Erythrocyte count (RBC)*	Mean corpuscular. volume (MCV)*
Platelet count*	Absolute reticulocytes
Activated partial thromboplastin time	Blood cell morphology
Prothrombin time	

* Recommended for combined chronic/carcinogenicity studies based on Guideline 870.4300.

b. Clinical chemistry:

ELECTROLYTES	OTHER
Calcium	Albumin*
Chloride	Creatinine*
Phosphorus	Urea nitrogen*
Potassium*	Total Cholesterol*
Sodium	Globulin and A/G ratio calculated
	Glucose*
	Total bilirubin
ENZYMES (more than 2 hepatic enzymes eg., *)	Total protein (TP)*
Alkaline phosphatase (ALK)*	Triglycerides
Alanine aminotransferase (ALT/also SGPT) *	Total bile acids
Aspartate aminotransferase (AST/also SGOT)*	Gama glutamyl transferase

* Recommended for combined chronic/carcinogenicity studies based on Guideline 870.4300.

c. Urinalysis:

Appearance*	Glucose
Volume*	Ketones
Specific gravity/osmolality*	Bilirubin
pH*	Blood/blood cells*
Sediment (microscopic)	Nitrate
Protein*	Urobilinogen

* Recommended for combined chronic/carcinogenicity studies based on Guideline 870.4300.

6. **Serological health screen:** A serological health screen for various virus types was conducted prior to dosing and at 6, 12, 18, and 24 months on 1 to 5 randomly selected animals/sex (as survival allowed) using sentinel animals euthanized for this purpose. Gross lesions were recorded. No tissues were saved. Any sentinel animal that was found dead or euthanized *in extremis* had a gross necropsy and gross lesions were saved for possible histopathologic evaluation.

7. **Plasma concentration of test substance and metabolites**: On test Day 345, blood was collected from 10 animals/sex/group (12-month satellite animals) for possible analysis of concentrations of triflumezopyrim and/or metabolites. Plasma was prepared and frozen but samples have not been analyzed.

8. **Sacrifice and pathology:** At termination, animals were sacrificed by isoflurane anesthesia followed by carbon dioxide inhalation and then exsanguinated. Ten animals/ sex/group were sacrificed at the interim period of 12 months and all surviving main study animals were sacrificed at the end of the 24-month period. Gross examinations were performed on all animals. Organs that were weighed are listed in Table 3. Organ weight/final body weight and organ weight/brain weight ratios were calculated. Tissues collected from animals receiving the highest dose (8000 ppm) and control (0 ppm) and from animals that died or were sacrificed prior to scheduled sacrifice were processed to slides and underwent histopathological evaluations. Gross lesions and suspected target tissues (testes for males, lung for females, and liver for both sexes at the interim necropsy; testes for males, uterus with cervix for females, and liver and lung for both sexes at the terminal necropsy), as determined by examination of the control and high dose animals, were processed to slides and examined microscopically for all animals.

Table 3. Combined chronic toxicity/carcinogenicity study in rats fed triflumezopyrim.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue	X	Aorta, thoracic*	X	Brain (multiple sections)*+
X	Salivary glands*	X	Heart*+	X	Periph nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	X	Spleen*+	X	Eyes (retina, optic nerve)*
X	Jejunum*	X	Thymus	X	GLANDULAR
X	Ileum*			X	Adrenal gland*+
X	Cecum*	X	UROGENITAL	X	Lacrimal gland
X	Colon*	X	Kidneys*+	X	Parathyroids*
X	Rectum*	X	Urinary bladder*	X	Thyroids*
X	Liver*+	X	Testes*+	X	OTHER
	Gall bladder* (not rat)	X	Epididymides*+	X	Bone (sternum and/or femur)
	Bile duct (rat)	X	Prostate*	X	Skeletal muscle
X	Pancreas*	X	Seminal vesicle*	X	Skin*
X	RESPIRATORY	X	Ovaries*+	X	All gross lesions and masses*
X	Trachea*	X	Uterus*+		
X	Lung*++a	X	Mammary gland*		
X	Nose*				
X	Pharynx*				
X	Larynx*				

* Required for combined chronic/carcinogenicity studies based on Guideline 870.4300.
+Organ weight required in combined chronic/carcinogenicity studies.
++Organ weight required if inhalation route.
[a] Lungs for animals in the 100, 500, and 2000 ppm dosage groups were microscopically examined for females only at the interim sacrifice and for both sexes at the terminal sacrifice.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** No treatment-related clinical signs of toxicity were observed. A greater number of females at the 8000 ppm group were noted to be thin in appearance

relative to controls, which correlated with the marked reductions in body weight that occurred in these animals.

2. **Mortality:** Treatment had no effect on survival in either sex at any dose level (Table 4).

Table 4. Survival Data: Combined Chronic Toxicity/Carcinogenicity Study in Rats Fed Triflumezopyrim.

Test Group	Concentration in diet (ppm)[a]	No. of Animals (Males)	No. of Animals (Females)
1	0	23/70	18/70
2	100	17/70	15/70
3	500	16/70	24/70
4	2000	18/70	20/70
5	8000	28/70	19/70

Table taken from page 38 of the study report (MRID 49382173).
[a] An interim necropsy was conducted on 10 animals/sex/concentration at 1 year.

B. BODY WEIGHT AND BODY WEIGHT GAIN:

Treatment had no adverse effects on body weight (Table 5) or body weight gain (Table 6) of male or female rats at 100 ppm or in males at 500 ppm. In females at 500 ppm, final (Week 103) mean body weight and overall (Weeks 1-103) body weight gain were 11.6 and 15.9% below control, respectively (both statistically significant). However, this was apparently due to a transient decrease in body weight gain over the approximately 12 to 18-month interval. During this interval (Days 357-539), mean body weight gain was 22.7% below control. However, over earlier intervals (3 and 12 months), and over the last 6 months of the study (Days 539-721), mean body weight gain in this group was comparable to control. The reason for this transient lower body weight gain is not known, but it was also seen in other male and female groups during this same general time interval. Since the period of lower body weight gain was transient, differences during most intervals were not statistically significant, and no other evidence of toxicity was observed in this group, these differences in 500 ppm dose group females were considered non-adverse. Triflumezopyrim caused significant decreases in body weight and body weight gain in both sexes at 2000 and 8000 ppm dose groups, with females more severely affected than males exposed to the same dietary concentration.

Table 5. Mean Body Weights (g): Combined Chronic Toxicity/Carcinogenicity Study in Rats Fed Triflumezopyrim.

	0 ppm	100 ppm	500 ppm	2000 ppm	8000 ppm
Males					
	0 mg/kg/day	**3.03 mg/kg/day**	**15.92 mg/kg/day**	**70.55 mg/kg/day**	**283.83 mg/kg/day**
Week 1	283.6±16.0	283.9±16.6	283.2±15.6	280.7±15.7	259.4±15.0[b] (-9%)
Week 13	577.7±55.4	574.0±59.0	576.6±50.0	557.4±49.0[a] (-3%)	495.8±44.2[b] (-14%)
Week 25	682.6±69.9	684.8±70.1	679.7±65.2	650.1±59.8[b] (-5%)	574.4±53.9[b] (-16%)
Week 51	816.2±94.2	820.4±95.0	803.8±93.9	760.5±78.2[b] (-7%)	654.1±63.2[b] (-20%)
Week 77	893.9±126.0	903.2±110.7	854.3±142.7	784.9±118.0[b] (-12%)	676.4±82.5[b] (-25%)
Week 103	830.7±151.3	884.6±127.4	856.3±122.1	769.9±121.8[b] (-8%)	669.0±95.4[b] (-19%)
Females					
	0 mg/kg/day	**3.23 mg/kg/day**	**17.34 mg/kg/day**	**73.80 mg/kg/day**	**395.88 mg/kg/day**
Week 1	190.7±12.5	189.8±11.8	189.2±11.9	185.2±10.7[b] (-3%)	168.2±10.2[b] (-12%)
Week 13	312.1±24.8	308.0±24.7	306.1±25.6	287.2±19.4 (-8%)	258.4±17.8[b] (-17%)
Week 25	351.0±33.6	346.6±32.7	341.3±34.3	314.2±22.3[b] (-11%)	278.3±20.1[b] (-21%
Week 51	451.3±65.4	449.1±63.9	426.9±67.0	370.6±43.0[b] (-18%)	303.7±28.1[b] (-33%)
Week 77	519.5±85.7	523.7±81.3	473.8±84.9[b]	410.6±60.3[b] (-21%)	314.5±37.0[b] (-40%)
Week 103	589.8±114.4	576.4±71.6	521.6±102.3[a] (-12%)	454.0±80.5[b] (-23%)	321.5±45.7[b] (-45%)

Data taken from pages 150-170 of the study report (MRID 49382173).

[a] Significantly different from control by Welch's t-test criteria, p <0.05.
[b] Significantly different from control by Welch's t-test criteria, p <0.01.

Table 6. Mean body weight gains (g): Combined chronic toxicity/carcinogenicity study in rats fed Triflumezopyrim.

	0 ppm	100 ppm	500 ppm	2000 ppm	8000 ppm
Males					
	0 mg/kg/day	**3.03 mg/kg/day**	**15.92 mg/kg/day**	**70.55 mg/kg/day**	**283.83 mg/kg/day**
Week 1–13	343.4	341.4	343.6	322.7[a] (-6%)	262.2[b] (-24%)
Week 13–25	103.9	110.8	103.1	93.7[a] (-10%)	78.7[b] (-24%)
Week 1–51	581.2	587.4	571.3	526.1[b] (-10%)	420.2[b] (-28%)
Week 51–77	73.8	76.1	48.3 (-35%)	32.4[a] (-56%)	20.7[b] (-72%)
Week 77–104	-54.5	-15.4	-64.4	-65.1	-42.7
Overall body weight gain	579.3	630.2	607.3	513.3 [a] (-11%)	423.3[b] (-27%)
Females					
	0 mg/kg/day	**3.23 mg/kg/day**	**17.34 mg/kg/day**	**73.80 mg/kg/day**	**395.88 mg/kg/day**
Week 1–13	144.9	143.6	139.1	121.3[b] (-16%)	96.1[b] (-34%)
Week 13-25	39.0	38.6	35.2	26.9[b] (-31%)	20.0[b] (-49%)
Week 1–51	284.1	284.6	259.9	205.0[b] (-28%)	141.4[b] (-50%)
Week 51–77	66.9	67.0	51.7 (-22.7%)	41.3[a] (-38%)	9.9[b] (-85%)
Week 77–104	60.1	53.1	58.3	45.4 (-24%)	13.2[a] (-78%)
Overall body weight gain	422.6	412.6	355.4[a] (-16%)	288.0[b] (-32%)	162.8[b] (-62%)

Data taken from pages 182 and 193 of the study report (MRID 49382173).
[a] Significantly different from control by Welch's t-test criteria, p <0.05.
[b] Significantly different from control by Welch's t-test criteria, p <0.01.

C. FOOD CONSUMPTION AND COMPOUND INTAKE:

Treatment-related lower food consumption and/or food efficiency were seen during the study in males and females at ≥2000 ppm, with food efficiency more severely affected in females than in males exposed to the same dietary concentration (Table 7).

Table 7. Food consumption (g/animal/day) and food efficiency: combined chronic toxicity/carcinogenicity study in rats fed triflumezopyrim.

	0 ppm	100 ppm	500 ppm	2000 ppm	8000 ppm
Males					
	0 mg/kg/day	**3.03 mg/kg/day**	**15.92 mg/kg/day**	**70.55 mg/kg/day**	**283.83 mg/kg/day**
Food consumption, Week 1–13	25.45	25.52	25.05	24.65	21.48[a] (-5%)
Food consumption, Week 1–51	26.25	26.35	26.16	25.23	22.98[a] (-2%)
Food consumption, Week 1–104	26.34	26.23	25.97	25.85	22.90[a] (-3%)
Food efficiency (%), Week 1–13	12.86	12.61	13.02	12.45	12.27[a] (-4%)
Food efficiency (%), Week 1–51	5.75	5.73	5.56	5.27[a]	4.85[a] (-15%)
Food efficiency (%), Week 1–104	2.77	3.12	2.90	2.51	2.40 (-14%)
Females					
	0 mg/kg/day	**3.23 mg/kg/day**	**17.34 mg/kg/day**	**73.80 mg/kg/day**	**395.88 mg/kg/day**
Food consumption, Week 1–13	17.04	16.83	16.60	15.76[a] (-7.5%)	13.95[a] (-20%)
Food consumption, Week 1–51	17.85	17.69	17.37	16.43[a] (-8%)	15.70[a] (-12%)
Food consumption, Week 1–103	18.03	18.06	17.79	16.84[b] (-7%)	16.06[a] (-11%)
Food efficiency (%), Week 1–13	7.90	7.80	7.80	7.20[a] (-9%)	7.27[a] (-8%)
Food efficiency (%), Week 1–51	4.08	4.11	3.82[b] (-6%)	3.16[a] (-22%)	2.47[a] (-39%)
Food efficiency (%), Week 1–103	2.92	2.92	2.60	2.30[a] (-12%)	1.38[a] (-53%)

Data taken from pages 198, 193, 207 and 211 of the study report (MRID 49382173).
[a] Significantly different from control by the Welch's t-test criteria, $p < 0.01$
[b] Significantly different from control by the Welch's t-test criteria, $p < 0.05$

Food consumption values in males at 2000 ppm and below and in females at 500 ppm and below were comparable to control throughout the study. Mean food consumption in 8000 ppm males was 12.5% and 13.1% below control over one and two years, respectively (both statistically significant). In females, mean food consumption at 8000 ppm was 12% and 11% below control over one and two years (both statistically significant). Mean food consumption at 2000 ppm was 8% and 6.7% below control over one and two years (both statistically significant). Food consumption effects at both concentrations in females were considered test substance-related.

Food efficiency effects generally correlated with body weight gain effects. Food efficiency was significantly lower during the study in males and females at ≥2000 ppm, with females more severely affected than males exposed to the same dietary concentration. In 8000 ppm males, mean food efficiency over the first year and over the entire study was 15.7 and 13.4% below control, respectively. In 2000 ppm males, mean food efficiency over the first year and over the entire study was 8.3 and 9.4% below control, respectively. Only the one-year interval difference was statistically significant at both concentrations. In 8000 ppm females, mean food efficiency over the first year

and over the entire study was 39.5 and 52.7% below control, respectively. In 2000 ppm females, mean food efficiency over the first year and over the entire study was 22.5 and 21.2% below control, respectively. All differences in these female groups were statistically significant, and all were considered treatment-related.

D. OPHTHALMOSCOPIC EXAMINATIONS:

No treatment-related ophthalmological observations were seen at any dose level.in either males or females.

E. CLINICAL PATHOLOGY:

1. **Hematology and clinical chemistry:** No treatment-related adverse effects were seen in any of the hematological parameters at any dose in either sex. The mild alterations observed during the first year of the study (decreases in red cell mass and eosinophils in both sexes at 8000 ppm) were not considered to be adverse based on the low magnitude of change, no associated increases in mean cell volume or reticulocyte count, lack of corroborative histopathological lesions in the hematopoietic system, and the absence of similar alterations at termination.

   There were no treatment-related effects at the 24-month interval on differential white blood cell (WBC) counts obtained from animals exposed to 8000 ppm. No test substance-related effects on WBC differential counts were observed in any treatment group among samples collected from *in extremis* animals.

   There was no adverse treatment related changes in any of the clinical chemistry parameters at any dose. The minor alterations observed in some of the parameters during the first year of the study were: mild increases in urea nitrogen and phosphorus in both sexes at 8000 ppm; mild decreases in chloride in both sexes at 8000 ppm; mild increases in cholesterol in females at 2000 and 8000 ppm; and increases in bile acids in females at 8000 ppm. These findings were considered non-adverse due to the low magnitude of changes and the absence of corroborative histopathological lesions in the appropriate organ systems.

2. **Urinalysis:** There were no treatment-related changes in urine parameters in male or female rats.

3. **Coagulation:** There were no treatment-related changes on coagulation in male or female rats.

F. SEROLOGICAL HEALTH SCREEN:

All serological health screen results were negative for all the intervals.

G. SACRIFICE AND PATHOLOGY:

1. **Organ weight:** At the interim sacrifice, treatment-related changes in liver weights were seen in both sexes. At the terminal sacrifice, treatment-related increased organ weights were observed in the liver and uterus of females.

Table 8. Organ weights: Combined Chronic Toxicity/Carcinogenicity Study in Rats Fed Triflumezopyrim.

Parameter	0 ppm	100 ppm	500 ppm	2000 ppm	8000 ppm
12-Month (Interim) - Males					
	0 mg/kg/day	**3.03 mg/kg/day**	**15.92 mg/kg/day**	**70.55 mg/kg/day**	**283.83 mg/kg/day**
Absolute liver weight (g)	21.562 ±3.59	21.186±3.72	21.591±4.77	23.793±5.39 (+10%)	22.822±2.53 (+6%)
Relative[a] liver weight (%)	2.8972±0.42	2.7690±0.24	2.8122±0.44	3.3905±0.84[d] (+17%)	3.6161±0.23[b] (+25%)
Liver to brain weight (%)	9.2124±1.20	9.2311±1.49	9.2387±1.94	10.3497±2.29 (+12%)	9.6363±1.13 (+5%)
12-Month (Interim) - Females					
	0 mg/kg/day	**3.23 mg/kg/day**	**17.34 mg/kg/day**	**73.80 mg/kg/day**	**395.88 mg/kg/day**
Absolute liver weight (g)	10.640±1.16	10.799±1.38	11.534±1.92	10.878±1.62 (+2%)	10.845±0.79 (+2%)
Relative[a] liver weight (%)	2.9035±0.37	2.6723±0.15	2.9162±0.28	3.3187±0.32[b] (+14%)	3.8129±0.23[b] (+31%)
Liver to brain weight (%)	5.0733±0.62	5.2075±0.65	5.3823±0.92	5.2982±0.76	5.2304±0.40
24-Month (Terminal)-Females					
	0 mg/kg/day	**3.23 mg/kg/day**	**17.34 mg/kg/day**	**73.80 mg/kg/day**	**395.88 mg/kg/day**
Absolute liver weight (g)	14.279±3.06	14.028±2.75	13.163±2.81	13.019±2.01	13.100±2.63
Relative[a] liver weight (%)	2.6496±0.42	2.5776±0.35	2.7063±0.42	3.1189±0.51[c] (+18%)	4.4074±0.73[b] (+66%)
Liver to brain weight (%)	6.9143±1.66	6.7498±1.18	6.3713±1.31	6.3450±0.88	6.5204±1.29
Absolute uterus weight (g)	1.129±0.33	1.210±0.72	1.100±0.39	1.513±0.87	4.568±8.59[c] (+404%)
Relative uterus weight (%)	0.2221±0.09	0.2296±0.14	0.2324±0.09	0.3630±0.21	1.5571±2.95[c] (+701%)
Uterus to brain weigh (%)	0.5459±0.16	0.5862±0.35	0.5353±0.19	0.7409±0.43	2.2805±4.36[b] (+417%)

Data taken from pages 1607-1647 of the study report (MRID 49382173).
[a] Relative weight is defined as the organ to body weight ratio.
[b] Significantly different from control by the Welch's t-test criteria, p <0.01.;
[c] Significantly different from control by the Welch's t-test criteria at p<0.05

At the interim sacrifice, treatment-related increased relative liver weights were observed in males (17.03%) and females at 2000 ppm (14.30% increase), and in males (24.81% increase) and females (31.32% increase) at 8000 ppm. At the terminal sacrifice, treatment-related increased organ weights were observed in the liver and uterus of females. In females, mean relative liver weight was statistically significantly increased at 2000 ppm (increased 17.71%) and at 8000 ppm (increased 66.34%). These weight increases correlated with centrilobular hepatocellular hypertrophy at 2000 ppm (females only) and 8000 ppm (both sexes).

Mean uterus weights were statistically significantly increased (mean absolute [304.61%], and relative to both body [601.08%] and brain [317.75%] weights) at the 8000 ppm dose level; however, there was high variability noted at this dose. Most of the uterine weight increase was due to dilatation of the uterine horns in one animal due to a malignant schwannoma. Uterus weights at 2000 ppm were not statistically significant and had a large standard deviation due to a single animal with dilatation of the horns, one animal with stromal sarcoma, and one animal with a stromal polyp.

2. **Gross pathology:** No test substance-related gross lesions were observed at necropsy at the interim sacrifice. At the terminal sacrifice, treatment-related macroscopic observations were noted in the lung of male and female rats and the uterus of females. In the lung, there was an increased incidence of focus/foci, tan and white, at 8000 ppm in both sexes and at 2000 ppm in females only. In the uterus of females, there was an increased

incidence of enlarged uterus at 2000 and 8000 ppm. Enlarged uterus was correlative to the presence of tumors at 8000 ppm, and to dilatation of the uterus with or without endometrial hyperplasia, inflammation, or hemorrhage.

3. **Histopathology:** At the interim sacrifice, treatment-related lesions were seen in the liver of both males and females at 8000 ppm and in the testes of males at 8000 ppm (Tables 9 and 10). In the liver, centrilobular hepatocellular hypertrophy of minimal severity was noted in all ten of the animals at 8000 ppm in both sexes. Hypertrophy was characterized by minimal enlargement of hepatocytes surrounding central veins. In the testes, an increase in the incidence of minimal interstitial cell hyperplasia was observed at 8000 ppm. Hyperplasia was characterized by small nodular foci of interstitial cells. A slight increase in alveolar histiocytosis of the lung (primarily scored as minimal) was noted in the 8000 ppm females.

   At the terminal sacrifice, treatment-related non-neoplastic lesions were seen in the liver, lung, testes, and uterus. Males at 2000 ppm had an increase in the incidence of bile duct hyperplasia (63% vs. 47% in controls; minimal to mild severity), while only a slight increase (7%) in hepatocellular hypertrophy was observed in females at this dose. At 8000 ppm, males also had an increase in the incidence of focal cystic degeneration (41% vs. 24% in controls; minimal to mild severity), bile duct hyperplasia (77% vs. 47% in controls; minimal to mild severity) and centrilobular hypertrophy (10% vs. 0% in controls; minimal severity). Females at this dose developed individual hepatocyte necrosis (4% vs. 0% controls) and minimal to mild centrilobular hepatocellular hypertrophy (67% vs. 0% controls).

   In the testes of males, there was a statistically significant increase in incidence of interstitial cell hyperplasia at 8000 ppm at terminal sacrifice (23% vs. 4% in controls; minimal to mild severity); however, this was not associated with an increase in interstitial cell tumors.

   In the lung, there was a treatment-related increase in incidence and severity (minimal to mild) of alveolar histiocytosis in 2000 ppm and 8000 ppm females at terminal sacrifice. This lesion was not considered adverse given the low severity. Additionally, a treatment-related increase was not observed in males, the lesions were seen in control animals, the lesion is commonly observed in aging rats, and there were no other treatment-related findings in the lung observed in this study.

   Increased incidences of uterine lesions were observed in females at 8000 ppm. Lesions included dilation/inflammation/hyperplasia (9% vs. 0% in controls; severity ranged from minimal to severe), cystic endometrial hyperplasia (11% vs. 1% in controls; minimal to mild severity), and squamous cell hyperplasia (9% vs. 3% in controls; minimal to mild severity). In some instances, the uterus was dilated with areas of inflammation and areas of endometrial epithelium hyperplasia, while in other instances there was cystic endometrial hyperplasia with a more generalized proliferation of endometrial epithelium.

Table 9. Non-neoplastic lesions in male rats in the combined chronic toxicity / carcinogenicity study in rats fed triflumezopyrim.

	0 ppm	100 ppm	500 ppm	2000 ppm	8000 ppm
	0 mg/kg/day	3.03 mg/kg/day	15.92 mg/kg/day	70.55 mg/kg/day	283.83 mg/kg/day
12-Month (Interim)					
Liver					
Hypertrophy, hepatocyte, centrilobular	0/10[a]	0/10	0/10	0/10	10/10 [b]

	0 ppm	100 ppm	500 ppm	2000 ppm	8000 ppm
	0 mg/kg/day	3.03 mg/kg/day	15.92 mg/kg/day	70.55 mg/kg/day	283.83 mg/kg/day
12-Month (Interim)					
Testes					
Hyperplasia, interstitial cell	0/10	0/10	0/10	0/10	6/10 [d,e]
Lung					
Histiocytosis, alveolar	4/10	ne[c]	ne[c]	ne[c]	5/10
Minimal	4				4
Mild	0				1
24-Month (Terminal)					
Liver					
Degeneration, cystic, focal	17/70 (24%)	15/70 (21%)	17/70 (24%)	19/70 (27%)	29/70[d,e] (41%)
Hyperplasia bile duct	33/70 (47%)	31/70 (44%)	33/70 (47%)	44/70 (63%)	54/70[b,e] (77%)
Hypertrophy, hepatocyte, centrilobular	0/70	0/70	0/70	0/70	7/70[d,e] (10%)
Testes					
Hyperplasia, interstitial cell	3/70 (4%)	7/70	8/70	8/70	16/70[b,e] (23%)
Lung					
Histiocytosis, alveolar	30/70	21/70	14/70[b]	22/70	37/70
Minimal	28	21	14	19	26
Mild	2	0	0	3	11

Data taken from pages 1648-1835 of the study report (MRID 49382173).
[a] Number of organs with microscopic change/number of animals examined.
[b] Statistically significant by the Fisher's exact criteria, $p < 0.01$.
[c] Not evaluated.
[d] Statistically significant by the Fisher's exact criteria, $p < 0.05$.
[e] Statistically significant by the Cochran-Armitage criteria, $p < 0.05$.

Table 10. Non-neoplastic lesions in female rats in the combined chronic toxicity / carcinogenicity study in rats fed triflumezopyrim.

	0 ppm	100 ppm	500 ppm	2000 ppm	8000 ppm
	0 mg/kg/day	3.23 mg/kg/day	17.34 mg/kg/day	73.80 mg/kg/day	395.88 mg/kg/day
12-Month (Interim)					
Liver					
Hypertrophy, hepatocyte, centrilobular	0/10[a]	0/10	0/10	0/10	10/10[b,d]
Lung					
Histiocytosis, alveolar	3/10	2/10	2/10	4/10	8/10
Minimal	2	2	2	3	6
Mild	1	0	0	1	2
24-Month (Terminal)					
Liver					
Necrosis, individual hepatocyte	0/70	0/70	0/70	0/70	3/70[b] (4%)
Hypertrophy, hepatocyte, centrilobular	0/70	0/70	0/70	5/70[b] (7%)	47/70[b,c] (67%)
Uterus					
Dilation/inflammation/hyperplasia	0/70	1/70	0/70	0/70	6/70[b,d] (9%)
Hyperplasia, cystic endometrial	1/70 (1%)	0/70 (0%)	1/70 (1%)	2/70 (3%)	8/70[b,d] (11%)
Hyperplasia, squamous cell	2/70 (3%)	0/70 (0%)	3/70 (4%)	1/70 (1%)	6/70 (9%)
Lung					
Histiocytosis, alveolar	25/70	16/70	17/70	37/70	52/70[b,c]
Minimal	24	13	14	22	17
Mild	1	3	3	15	32
Moderate	0	0	0	0	3

Data taken from pages 1648-1835 of the study report (MRID 49382173).
[a] Number of organs with microscopic change/number of animals examined.
[b] Statistically significant by the Cochran-Armitage criteria, $p < 0.05$.
[c] Statistically significant by the Fisher's exact criteria, $p < 0.01$.
[d] Statistically significant by the Fisher's exact criteria, $p < 0.05$.

Table 11. Neoplastic Lesions in Female Rats: in the Combined Chronic Toxicity / Carcinogenicity Study in Rats Fed Triflumezopyrim.

	0 ppm	100 ppm	500 ppm	2000 ppm	8000 ppm
	0 mg/kg/day	3.23 mg/kg/day	17.34 mg/kg/day	73.80 mg/kg/day	395.88 mg/kg/day
Number Examined	70	70	70	70	70
Uterus					
Carcinoma, squamous cell	0[a] (0%)	0	0	1 (1.4%)	5[b,c] (7.1%)
Adenocarcinoma,	0 (0%)	0	0	0	2[b] (2.86%)
Granular cell	0	1	1	0	4[b, c] (5.71%)
Liver					
Hepatocellular adenoma	0	0	0	0	2 (2.86%)[b]

Data taken from pages 1928-1971 of the study report (MRID 49382173).
[a] Number of organs with microscopic change.
[b] Statistically significant by the Cochran-Armitage criteria, $p < 0.05$.
[c] Statistically significant by the Peto test.

There were no increases observed in any tumor type among males. In females, there was an increase in squamous cell carcinomas, adenocarcinomas, and granular cell tumors in the uterus at 8000 ppm (Table 11). There was also a slight increase in the incidence of hepatocellular adenomas in females at this dose (2/70 vs. 0/70 in controls).

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

The NOAEL was 500 ppm (15.92 mg/kg day) for male rats and 500 ppm (17.34 mg/kg bw/day) for female rats. This NOAEL was based on reductions in body weight and nutritional parameters, and clinical pathology and anatomic pathology findings in males and females at the 2000 ppm concentration. Under the conditions of this study, triflumezopyrim was not oncogenic at dietary concentrations up to 8000 ppm for male rats and up to 2000 ppm for female rats. Test substance-related neoplastic findings were limited to females at 8000 ppm and occurred at an exposure level that produced marked reductions in absolute body weight that greatly exceeded the maximum tolerable dose (MTD).

B. REVIEWER COMMENTS:

Significant decreases in absolute body weights were seen at 2000 ppm (females only) and 8000 ppm (both sexes). Relative (not absolute) liver weights were increased in males and females at 2000 and 8000 ppm dose groups at the 1-year interim sacrifice and only in females at termination at these dose levels. At 2000 ppm, only females had an increased incidence of hepatocellular hypertrophy at terminal sacrifice. At 8000 ppm, increased incidence of hepatocellular hypertrophy was observed in both sexes. In males, there was a treatment-related increase in the incidence of bile duct hyperplasia observed at 2000 ppm and 8000 ppm. Also seen in the liver at 8000 ppm were increases in focal cystic degeneration (males only) and individual cell necrosis (females only).

In addition to the liver observations, non-neoplastic lesions of the testes of male rats and uterus of female rats were observed at 8000 ppm. Interstitial cell hyperplasia of the testes was seen at termination in males at 8000 ppm (23% vs. 4% in controls), but was not associated with organ weight changes or interstitial cell tumors. Uterine lesions included dilation/inflammation/hyperplasia (9% vs. 0% in controls), cystic endometrial hyperplasia (11% vs. 1% in controls), and squamous cell hyperplasia (9% vs. 3% in controls).

There were no increases observed in any tumor type among males. In females, there was an increase in squamous cell carcinomas, adenocarcinomas, and granular cell tumors in the uterus at 8000 ppm. There was also a slight increase in the incidence of hepatocellular adenomas in females at this dose. It should be noted the Cancer Assessment Review Committee (CARC) concluded that the 8000 ppm dose was considered excessive for females based on excessive decreases in absolute body weight, body weight gains, and food efficiency (M. Wilson; 10-AUG-2017; TXR# 0050441).

The NOAEL is 500 ppm (15.92 mg/kg bw/day for male rats and 17.34 mg/kg bw/day for female rats).

The LOAEL is 2000 ppm (70.55 mg/kg bw/day for males and 73.80 mg/kg bw/day for females) based on significant decreases in absolute body weight in females and increased incidence of bile duct hyperplasia in males. At the highest dose tested (8000 ppm), decreased absolute body weights in both sexes, increased

relative liver weights in females, and additional histopathological lesions in the liver (both sexes), testes, and uterus were also observed.

C. STUDY DEFICIENCIES:

None

D. REFERENCES:

Giknis, M.L.A and Clifford, C.B (2013). Compilation of Spontaneous Neoplastic Lesions and Survival in Crl:CD(SD) Rats from Control Groups. March 2013, Charles River Laboratories.
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